Phytochemical Differences Between a Zygocactus Hybrid Cultivar and its Parental Types

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Zygocactus or "Christmas Cactus" exists in an array of horticultural varieties which exhibit a wide range of flower colors and cladophyll morphologies. Many cultivars are the result of successive hybridizations and attempts to produce new and better varieties. Characterization of the cultivars on the basis of phenological and morphological attributes has served for patent disclosures, but such features as flower color and size sometimes have proved to be inadequate. Other more accurate and objective measures of differences are needed for positive identification of similar cultivars.

Chemical evaluation or "plant fingerprinting" has been proposed as an adjunct to classical methods to describe plant cultivars--especially those to be protected by plant patent laws. Just as chemotaxonomy was used successfully in the solution of some difficult systematic problems where other cytological, anatomical and/or morphological approaches failed (Harborne, 1968), phytochemical evaluation could possibly be a more accurate way to distinguish between clonal horticultural varieties and document their parentage. The ancestry of a hybrid between two species has already been traced by examination of its chemical constituents (Smith and Levin, 1963). Recent studies have also shown that the flavonoid distributions differ between clonal hybrid horticultural varieties in both poinsettia (Asen, 1979) and roses (Asen, 1977). Many of the differences demonstrated were quantitative rather than qualitative, but the ancestry of the hybrid clones was still apparent.

Phytochemical evaluation was initiated to determine the flavonoid differences between a Zygocactus hybrid cultivar and its parental types. The variety selected for study was 'Gold Charm' because its exact parentage was known. 'Gold Charm' is a hybrid between a yellow diploid male and a white tetraploid female, and this variety is the first patented yellow flowered Christmas cactus.

Knowledge of flavonoid distribution in the Cactaceae is limited to only a small percentage of genera. As of 1979 only 33 out of 150 genera had been examined for flavonoids (Gornall, 1979) and few

appear to have been reported since. Betalains were found in 32 out of 33 examined, with no anthocyanidins present. Since then, genera examined for flavonoids include Echinocereus (Miller and Bohm, 1982) and Opuntia subgenus Cylindropuntia (Clark et al., 1980). These studies indicated that the flavonols are the predominant flavonoids in Cactaceae, with kaempferol, quercetin, isorhamnetin and their derivatives being the most common (Miller and Bohm, 1982). Our examination for flavonoids in Zygocactus represents the first isolation of these compounds for the genus and demonstrates the potential usefulness of phytochemical methods to distinguish three closely related horticultural varieties.

MATERIALS AND METHODS

Source of Materials: Samples of vegetative and floral material of three horticultural varieties of Zygocactus, Cobia 18950 hybrid, Cobia 1178 female parent and Cobia 15139 male parent, were collected from Cobia greenhouses in Winter Garden, Florida at the height of flowering season.

Sample Preparation: Vegetative samples, prior to solvent extraction, were separated from floral material, washed with distilled water and the cladophyll was sliced lengthwise with a clean razor blade to expose maximum surface area. Floral samples were prepared by removing the ovaries and sepals, so that only the corolla, stigma and style remained. Both floral and vegetative material were then dried rapidly at 40°C in a herbarium oven for several days and ground into small pieces with a mortar and pestle. Dried vegetative and floral samples (prior to storage in the dark) were weighed to 3.0 g and 1.0 g respectively.

Extraction of Flavonoids: Flavonoids were sequentially extracted from the dried plant material using solvents of increasing polarity. Hexane, 30 ml, was added to each sample and the sample was placed in the dark for 48 hr. Hexane extract was decanted and the extract was stored separately. The extraction procedure was repeated sequentially using absolute methanol and then equal parts of methanol and water. Each solvent extract was concentrated to a final volume of 3-4 ml. The hexane extraction step was subsequently eliminated after chromatographic analysis revealed no flavonoids present.

Preparative Paper Chromatography: Each concentrated extract was applied individually to Whatman 3MM chromatographic paper. Volume of extract applied to each chromatogram depended on the concentration of flavonoids in the sample, but typically ranged 0.125 to 0.250 ml. Chromatograms were developed using descending two-dimensional paper chromatography (Mabry, 1970). Tertiary butanol: glacial acetic acid: water (3:1:1 V/V/V) was used for first dimension separation (22-24 hr) and glacial acetic acid: water (15:85 V/V) was used for the second dimension separation (3-4 hr). Developed chromatograms were viewed for fluorescing or absorbing spots under long wave ultraviolet light with and without the presence of ammonia fumes.

Each spot represented a "partially purified" flavonoid and was described by color reaction and R_f values for both dimensions of chromatographic separation. A series of chromatograms was developed using three different flavonoid standards: quercetin, rutin and kaempferol. Each standard was applied in both a high concentration and a low concentration to separate 3MM Whatman sheets and the R_f values of the standards were determined after two-dimensional chromatography. The R_f values of these standards, as well as the flavonoid components of the floral and vegetative extracts, were redetermined from chromatograms cospotted with both a standard and the floral or vegetative extract. These R_f values were compared with the R_f values determined for these same floral and vegetative flavonoids using two-dimensional chromatography of extract alone; i.e., without a standard.

Acid hydrolysis: The extracts were hydrolyzed using 5 ml of equal parts of 2N HCL: 95% ethanol (1:1 V/V) to 1 ml of concentrated extract. The solutions were refluxed at 100°C for 4 hr. The resulting hydrolyzed extracts were spotted on Whatman 3MM chromatographic paper in approximately the same concentration as the original extract and developed two-dimensionally in the same manner as the unhydrolyzed extracts.

Purification and Analysis of Flavonoids by Thin Layer Chromatography: Flavonoids which appeared as separate spots on the two-dimensional chromatograms were extracted from paper by cutting out the area of each spot from approximately 30 separate chromatograms. The flavonoids were eluted from the paper by adding sufficient methanol to make a liquid slurry. Eluted flavonoids were decanted, placed in separate vials, concentrated under partial vacuum to 2-3 ml, and stored in the dark. Silica gel instant thin layer chromatography (ITLC, Gelman) was used to evaluate the purity of the flavonoids separated by paper chromatography. The concentrated flavonoids eluted from paper were applied to three separate sheets of silica gel and these were allowed to develop in three solvent systems of varying polarity: 1) toluene: chloroform: acetone (8:5:7 V/V/V); 2) acetone: benzene (1:3 V/V); and 3) acetone: benzene (1:1 V/V). After examination of ITLC chromatograms under ultraviolet light, those flavonoids which appeared pure, i.e., migrated in ITLC as one component, were stored in the dark for further analysis by spectrophotometry. Solvents which achieved the best separation were selected for further purification of those flavonoids which appeared to consist of more than one component. Reverse phase C18 thin layer chromatographic analysis using methanol: water: glacial acetic acid (70:25:5 V/V/V) was used as a final evaluation for the purity of those flavonoids which had appeared pure on silica gel chromatograms as well as for those flavonoid components which were separated further by ITLC. Flavonoids which still appeared pure using the C18 reverse phase thin layer analysis were selected for ultraviolet/visible spectral analysis.

Ultraviolet/Visible Spectral Analysis: Approximately 0.10 mg of purified flavonoid was added to 10 ml of spectral grade methanol. Samples were analyzed on a Beckman DB-G spectrophotometer equipped with a Sargent model SRL recorder using methods previously described by Mabry et al. (1970). The absorption spectrum for each of the purified flavonoid isolates in spectral grade methanol was recorded in 250 nm to 400 nm range before and after the addition of chemical shift reagents. The wavelength maxima for Band I and Band II were determined from the spectral profile of methanol along with any additional shifts and peaks in the spectrum. The ultraviolet spectra and the shifts obtained with the purified flavonoids were compared to reference spectra documented by Mabry et al. (1970). A preliminary identification of the flavonoid class was made based upon spectral data, color charcteristics, and R_f values.

RESULTS

Flavonoids contained in extracts from both vegetative and floral material of Zygocactus hybrid 18950, female parent 1178 and male parent 15139 separated into many spot components on two-dimensional chromatograms (Fig. 1A and B). The composite flavonoid patterns of vegetative extracts differed significantly from floral extracts and further purifications and analyses were therefore done separately for vegetative and floral material.

Table 1. Summary of chromatographic differences observed among 18950, 15139 and 1178 cladophyll extracts.

Spot	18950	15139	1178
11	P	A	A
14	P	A	A
20	P	A	P
25	P	P	A

P refers to presence of spot.
A refers to absence of spot.

Comparison of flavonoid patterns obtained revealed that not all component spots were present in all the three cultivars. Spots numbered 11, 14, 20, and 25 obtained from vegetative material (Fig. 1A) and spots numbered 3, 9, 10 and 11 obtained from floral material (Fig. 1B) are all present in the hybrid, but each of these is missing from either one or both parental plants (Table 1 and 2).

Component color reactions and R_f values for Zygocactus flavonoids separated by two-dimensional chromatography (Tables 3 and

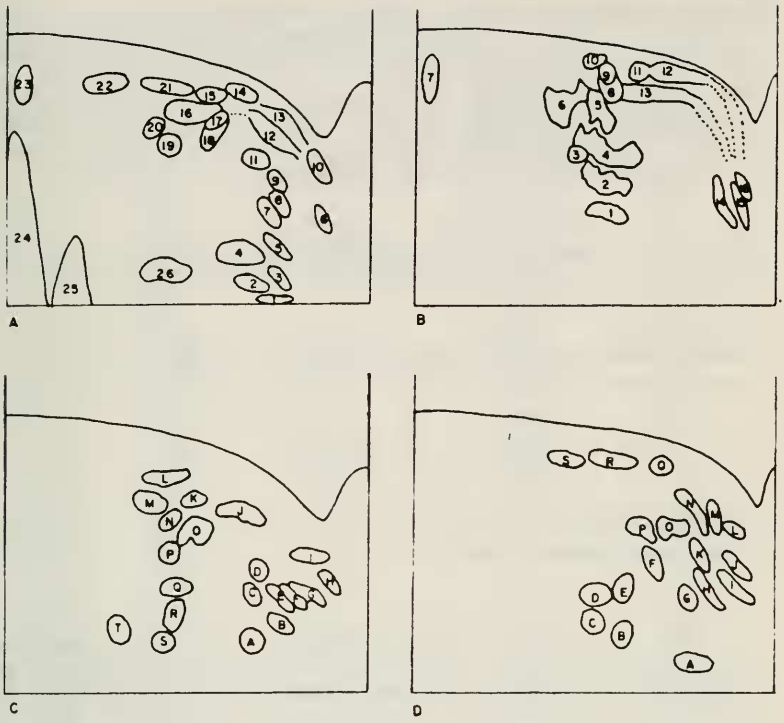

Figure 1. Chromatograms from Zygocactus extracts. A. The two-dimensional paper chromatographic composite pattern of flavonoids obtained from cladophyll extracts. B. The two-dimensional paper chromatographic composite pattern of flavonoids obtained from floral extracts. C. The two-dimensional paper chromatographic composite pattern of flavonoids obtained from acid hydrolyzed cladophyll extracts. D. The two-dimensional paper chromatographic composite pattern of flavonoids obtained from acid hydrolyzed floral extracts.

4) were compared to known flavonoids (Markham, 1982). We formulated a composite listing of all possible flavonoid classes and types which were found in the vegetative and floral material of Zygocactus (Table 5). Each numbered spot component was accordingly assigned to a particular class and type (Tables 6 and 7). The presence of 5-OH flavonoids (Markham, 1982) in vegetative spots numbered 11, 14 and 20 as well as floral spots numbered 1, 2 and 4 were detected by the color changes observed upon application of $AlCl_3$ spray reagent.

Table 2. Summary of chromatographic differences observed among 18950, 15139 and 1178 floral extracts.

Spot	18950	15139	1178
3	P	A	P
9	P	A	A
10	P	A	A
11	P	P	A

P refers to presence of spot.
A refers to absence of spot.

The flavonoid patterns obtained from two-dimensional chromatography of acid hydrolyzed extracts of Zygocactus were significantly different from the flavonoid patterns obtained from unhydrolyzed extracts for both vegetative (Fig. 1A and 1C) and floral (Fig. 1B and 1D) materials. Those flavonoid spot components which remained unchanged in color and R_f values following hydrolysis include vegetative spots numbered 6, 8 and 9 (Table 3) which were very similar to spots lettered H, E and D of the hydrolyzed vegetative extracts (Table 8), and floral spots numbered 15 and 16 (Table 4) which were similar to spots lettered I and J (Table 10) of hydrolyzed floral extracts. These spot components which remained unchanged are presumably the aglycones, i.e., flavonoids without a sugar linkage. Since the majority of flavonoids observed with two dimensional chromatography after acid hydrolysis changed R_f values and/or color reactions, most of the flavonoid compounds of Zygocactus probably are flavonoid glycosides.

Comparisons of the hydrolyzed flavonoid patterns among the cultivars revealed that their patterns differ. Component spots lettered A, S and T obtained from patterns of hydrolyzed vegetative material (Fig. 1C) and spots lettered A, F, K and Q obtained from patterns of hydrolyzed floral material (Fig. 1D) are all present in the hybrid, but each of these spot components is missing from either one or both parents (Tables 9 and 11).

Table 3. Color characteristics and R_f values of spots obtained from paper chromatography of cladophyll extracts.

Spot	R_f Values	Color UV	Color UV+NH_3	Color UV+$AlCl_3$
1	74,03	Yellow	I. Yellow	NG
2	68,10	-	F. Green	NG
3	75,14	Yellow	Yellow-Orange	NG
4	64,23	L. Purple	F. Green	NG
5	74,29	Yellow-Orange	NG	NG
6	87,53	-	D. Purple	NG
7	72,43	-	Green	NG
8	75,49	-	Blue	Blue-Violet
9	73,58	L. Blue	F. Blue	Blue-Violet
10	85,81	F. Green	I.F. Green	NG
11	68,63	-	-	Yellow
12	74,81	-	F. Green	NG
13	77,88	F. Green	NG	NG
14	65,92	Purple	D. Purple	I.F. Yellow
15	56,87	Blue	NG	NG
16	50,77	Green	NG	NG
17	58,77	Violet	NG	Blue
18	56,69	-	-	Blue-Green
19	44,64	Pink	NG	NG
20	41,70	-	-	Yellow
21	43,86	Blue-Green	NG	NG
22	27,84	Purple	NG	Blue
23	04,81	Yellow	NG	NG
24	04,34	Blue-Violet	F. Blue-Green	NG
25	18,13	Blue-Violet	F. Blue-Green	NG
26	44,15	Yellow-Green	NG	NG

R_f values are listed (1st dimension, 2nd dimension) x 100.
NG refers to no change in color.
I refers to intense.
F refers to fluorescent.
L refers to light.
D refers to dark.
- refers to no visible color.

Standards: Flavonoid standards of quercetin, rutin and kampferol showed little variation in R_f values and spot color when spotted alone or in combination with a plant extract and chromatographed two-dimensionally. The flavonoid standards were within ±5% of the published R_f values for the compounds (Fig. 2A, B, C). All other flavonoid components of vegetative and floral extracts showed no apparent variation in R_f values and color appearance when co-chromatographed with the standards.

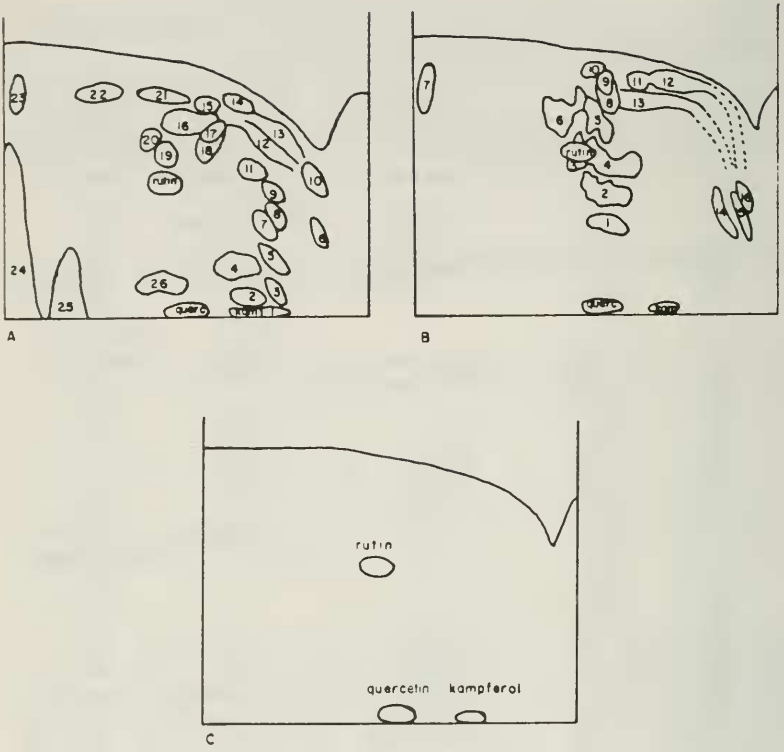

Figure 2. Chromatograms from Zygocactus extracts plus standards. A. Chromatographic pattern of standards plus vegetative extract. B. Chromatographic pattern of standards plus floral extracts. C. Chromatographic pattern of standards rutin, quercetin and kampferol.

Table 4. Color characteristics and R_f values of spots obtained from paper chromatography of floral extracts.

Spot	R_f Values	Color UV	Color UV+NH_3	Color UV+$AlCl_3$
1	52,36	L. Purple	NG	F. Yellow
2	50,48	D. Purple	Orange-Green	F. Yellow
3	44,57	-	Orange	NG
4	49,61	D. Purple	Orange-Green	F. Yellow
5	50,75	Blue-Green	NG	NG
6	41,76	Green	NG	NG
7	03,82	Yellow	NG	NG
8	53,80	L. Green	D. Green	Yellow
9	51,88	Yellow-White	NG	NG
10	49,91	D. Purple	NG	NG
11	62,93	Blue	I.F. Blue	NG
12	73,95	L. Blue	NG	NG
13	61,85	Green	F. Green	NG
14	84,46	-	F. Green	NG
15	90,48	D. Purple	NG	NG
16	91,84	-	I.F. Blue	NG

R_f values are listed (1st dimension, 2nd dimension) x 100.
NG refers to no changes in color.
I refers to intense.
F refers to fluorescent.
L refers to light.
D refers to dark.
- refers to no visible color.

Evaluation of Purity of Isolates: Some concentrated single spot isolates separated into more than one component when analyzed in one or more of the three solvent systems. Vegetative spots 1, 16, 21 and 25 and floral spots 4 and 11 purified by ITLC silica gel chromatography and further evaluated by C18 reverse phase chromoatography were apparently pure when analyzed by ultraviolet-visible spectrophotometry.

Spectral Data: Spectral data measurements using six diagnostic conditions, values for Band I and Band II for each spot component and the flavonoid class represented by those measurements were summarized (Table 12). The methanol spectral profile of the isolates from which measurements of Band I and Band II were also compared (Fig. 3A, B, C, D and 4A, B). A close correlation was seen between the spectral data assignment of flavonoid classes of the spots examined with the

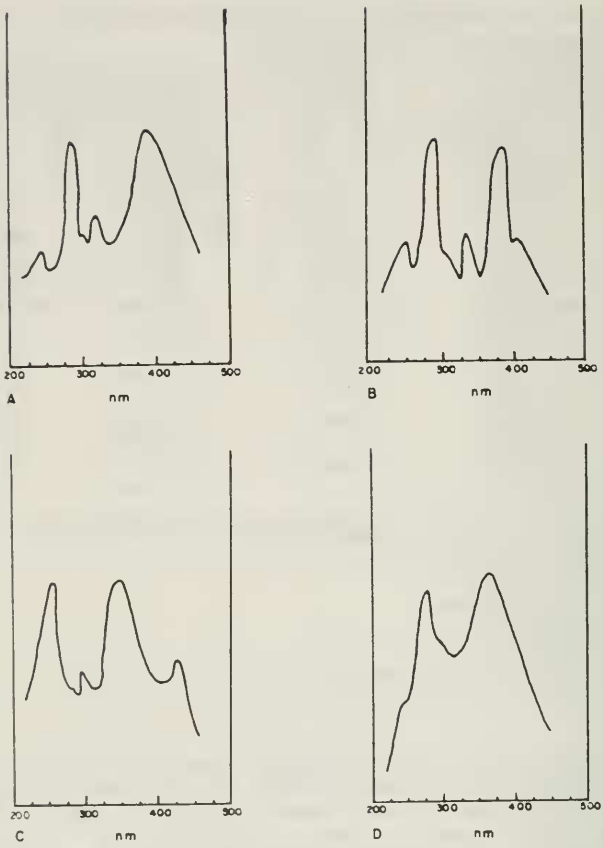

Figure 3. Ultraviolet/Visible methanol spectra of purified flavonoid isolates. A. Vegetative spot 1. B. Vegetative spot 16. C. Vegetative spot 21. D. Vegetative spot 25.

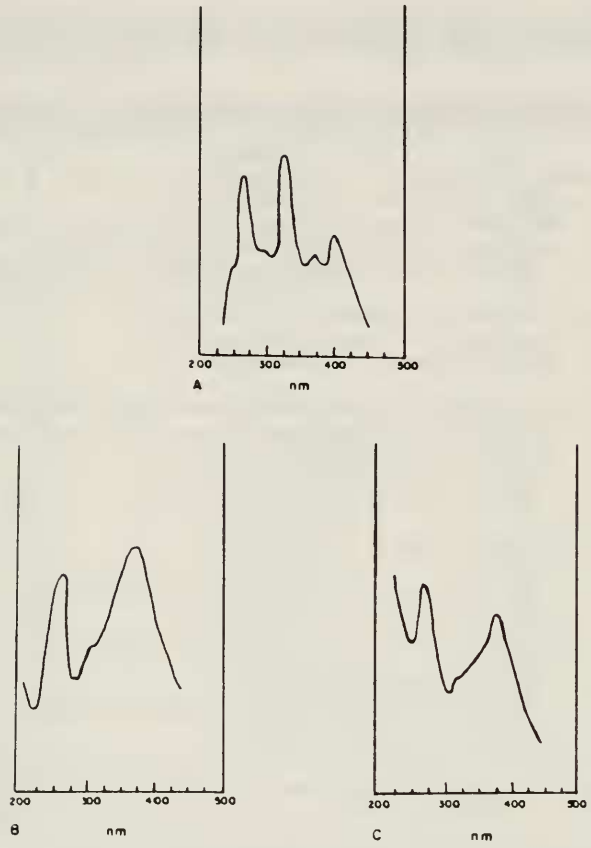

Figure 4. Ultraviolet/Visible methanol spectra of purified flavonoid isolates and rutin. A. Floral spot 11. B. Floral spot 4. C. Rutin.

Table 5. Flavonoid classes and types based on color and R_f values of spots obtained from chromatography of plant extracts of Zygocactus.

1. Commonly 5-OH flavones or flavonols 3-O-substituted with 4'-OH.
   A. Flavonol 3-O-diglycoside or flavonol 3-O-monoglycoside.

2. Commonly flavones or 3-O-substituted flavonols with 5-OH, but lacking a free 4'-OH.
   A. Flavonol 3-O-monoglycoside, 7-O-diglycoside, flavonol 3-O-diglycoside or flavonol 3, 7-O-diglycoside.
   B. Flavonol 3-O-diglycoside or flavonol 3-O-monoglycoside.

3. Dihydroflavonols.
   A. Aglycone.
   B. Dihydroflavonol 3-O-monoglycoside.

4. Flavonols lacking a free 5-OH, but with the 3-OH substituted.
   A. Flavonol 3-O-diglycoside or flavonol 3-O-monoglycoside.
   B. Flavonol 7-O-monoglycoside.
   C. Flavonol 7-O-diglycoside.
   D. Flavonol tri-O-glycoside.

5. Isoflavones lacking a free 5-OH.
   A. Aglycone.
   B. Isoflavone 7-O-diglycoside or 7-O-monoglycoside.

6. Flavonols with a free 3-OH and with or without a free 5-OH.
   A. Aglycone.
   B. Flavonols 7-O-monoglycoside.
   C. Flavonol 3-O-monoglycoside or 3-O-diglycoside.
   D. Flavonol 3, 7-O-triglycoside.
   E. Flavonol 7-O-diglycoside.

previous preliminary assignment of those spots to a particular flavonoid class based upon the R_f values and colors (Tables 6 and 7). Floral spot 4 appeared to have the best resolution and the sharpest peaks. When compared to the spectral data of known flavonoids (Markham, 1982), the spectral data of floral spot 4 appeared to be almost identical to the flavonoid rutin (Fig. 4B, C). R_f values, color reaction, and other structural elucidation information for floral spot 4 (Table 13) were similar to that of rutin. Direct co-chromatography of the rutin standard with the mixtures of flavonoids contained in the floral extracts revealed that rutin and floral component 4 overlap in position on the two dimensional chromatograms.

Table 6. Assignment of cladophyll chromatographic spots to a probable flavonoid class and type based upon the characteristics summarized in Table 3.

Spot	Flavonoid Class	Flavonoid Type
1	Flavonol	6A
2	Flavonol	4A, 6A or 6B
3	Flavonol	6B
4	Flavonol	4A or 6B
5	Flavonol	6B
6	Dihydroflavonol	3A
7	Flavonol	6C
8	Isoflavone and Flavonol	5A
9	Isoflavone and Flavonol	5A
10	Flavonol	4B or 6C
11	Flavonol	6C
12	Isoflavone and Flavonol	4B, 5B or 6C
13	Flavonol	6C
14	Dihydroflavonol	3B
15	Isoflavone and Flavonol	5B
16	Flavonol	6C
17	Dihydroflavonol	3B
18	Flavonol	6C
19	Flavonol	6C
20	Flavonol	6C
21	Flavonol	6D
22	Flavonol	2A
23	Flavonol	6D
24	Flavonol	4C
25	Flavonol	4A
26	Flavonol	6B

Table 7. Assignment of floral chromatographic spots to a flavonoid class and type based upon the characteristics summarized in Table 4.

Spot	Flavonoid Class	Flavonoid Type
1	Flavonol	2A
2	Flavonol	1A
3	Flavonol	6C or 6E
4	Flavonol	1A
5	Flavonol	6C
6	Flavonol	6D
7	Flavonol	6D
8	Flavonol	6D
9	Flavonol	6D
10	Flavonol or Dihydroflavonol	2A or 3B
11	Isoflavone	5B
12	Isoflavone	5B
13	Flavonol	4A
14	Flavonol	6C
15	Dihydroflavonol	3A
16	Isoflavone	5A

Table 8. Color characteristics and R_f values of spots obtained from paper chromatography of acid hydrolyzed cladophyll extracts.

Spot	R_f Values	Color UV	Color UV+NH_3	Color UV+$AlCl_3$
A	69,23	Yellow	NG	NG
B	77,34	-	Green	NG
C	69,41	Blue	I.F. Blue	NG
D	70,54	Blue	I.F. Blue	NG
E	76,45	Blue	-	Blue-Violet
F	80,49	Yellow-Green	NG	NG
G	84,54	Blue-Violet	NG	NG
H	90,60	L. Purple	D. Purple	NG
I	84,80	Yellow	F. Yellow	NG
J	66,77	Yellow-Pink	NG	NG
K	52,78	Green	NG	NG
L	44,83	Blue	F. Blue	NG
M	40,73	Purple	NG	NG
N	45,69	-	-	Yellow-Green
O	53,66	Pink		F. PinkWhite
P	46,55	-	-	Purple
Q	47,42	Green	NG	NG
R	48,33	-	-	F. Blue-Green
S	44,18	Purple	NG	NG
T	32,23	-	-	Yellow

R_f values are listed (1st dimension, 2nd dimension) x 100.
NG refers to no change in color.
I refers to intense.
F refers to fluorescent.
L refers to light.
D refers to dark.
- refers to no visible color.

Table 9. Summary of chromatographic differences observed among 18950, 15139 and 1178 acid hydrolyzed cladophyll extracts.

Spot	18950	15139	1178
A	P	P	A
S	P	A	A
T	P	A	A

P refers to presence of spot.
A refers to absence of spot.

Table 10. Color characteristics and R_f values of spots obtained from paper chromatography of acid hydrolyzed floral extracts.

Spot	R_f Values	Color UV	Color UV+NH_3	Color UV+$ALCL_3$
A	76,14	-	-	Yellow
B	56,22	-	-	F. Blue-Green
C	47,27	Blue	NG	F. Blue-Green
D	45,48	Purple	NG	NG
E	56,40	Blue	NG	F. Blue-Green
F	64,50	Yellow	NG	NG
G	74,38	Blue-Violet	NG	NG
H	80,43	-	F. Blue-Green	NG
I	88,48	L. Purple	D. Purple	NG
J	89,58	Blue	NG	NG
K	79,54	-	Yellow-Orange	NG
L	87,74	Brown	NG	NG
M	82,75	Yellow	I.F. Yellow	NG
N	76,75	Yellow-Orange	NG	NG
O	70,66	L. Orange	Orange	NG
P	61,64	Pink	NG	NG
Q	66,90	Yellow	I.F. Yellow	NG
R	53,88	Blue-Green	F. Blue-Green	NG
S	40,88	Purple	NG	NG

R_f values are listed (1st dimension, 2nd dimension) x 100.
NG refers to no change in color.
I refers to intense.
F.refers to fluorescent.
L refers to light.
D refers to dark.
- refers to no visible color.

Table 11. Summary of chromatographic differences observed among 18950, 15139 and 1178 acid hydrolyzed floral extracts.

Spot	18950	15139	1178
A	P	A	A
F	P	A	A
K	P	A	A
Q	P	A	P

P refers to presence of spot.
A refers to absence of spot.

Table 12. Spectral data of purified flavonoids and corresponding flavonoid class based upon the location of Band I and II. (V-Vegetative and F-Floral).

Spot	Diagnostic Condition	Area of Peak Maximum	Band I	Band II	Flavonoid Class
V-1	1	386,314,293,280,246	386	280	Flavonol
	2	387,315,293,282sh,243			
	3	383,312,284,276,267,240			
	4	382,311,282sh,275,267sh,240			
	5	389,319,293sh,280			
	6	387,317,293,280			
V-16	1	395,371,326,300,280,248	371	280	Flavonol
	2	396,371,327,300,281,247			
	3	396,371,326,303sh,287,280			
	4	397,370,327,278			
	5	397,371,329,305sh,288,281			
	6	397,371,330,305sh,281,287			
V-21	1	405,340,287,280sh,261	340	261	Flavonol
	2	383,341,293,280,262			
	3	381,340,313,288,280,265			
	4	382,344,311,280			
	5	383,345,313,287,280sh,263			
	6	384,345,314,288,280sh,260sh			
V-25	1	332,288,280,244	332	273	Flavonol
	2	331,289,280,244			
	3	330,288,280,273,265,258,234			
	4	331,280			
	5	332,288,280,273,266sh			
	6	332,288,280,273,266sh			
F-4	1	260,264sh,301sh,362	362	260	Flavonol
	2	265,266sh,320,413			
	3	273,305sh,323,435			
	4	270,300,324,362sh,405			
	5	272,310,325,397			
	6	265,299,330,390			
F-11	1	392,370,321,297,265,250	321	265	Isoflavone
	2	392,370,321,297,281,265,250			
	3	394,368sh,322,299sh,281,265,250			
	4	393,368sh,323,287,280			
	5	393,370,323,301,289,281,253			
	6	394,372,324,302,289,281,253			

Diagnostic conditions: 1. Methanol 2. NaOMe 3. AlCl3 4. AlCl3/HCL 5. NaOAc 6. NaOAc/H_3BO_3

Table 13. A comparison of flavonoid characteristics between rutin and floral spot numbered 4.

Characteristics	Rutin	Floral Spot 4
Color UV	Dark Purple	Dark Purple
Color UV+NH_3	Orange-Green	Orange-Green
Color UV+$AlCl_3$	F. Yellow	F. Yellow
R_f Values	44,56	49,61
Glycosidic Linkage	Present	Present
5-OH Substitution	Present	Present
Band I	360	362
Band II	260	260

R values are listed (1st dimension, 2nd dimension) x 100.
F refers to fluorescent.

DISCUSSION

Zygocactus hybrid 'Gold Charm' and the two parental types, female 1178 and male 15139 were distinguishable phytochemically by the composite chromatographic flavonoid patterns of vegetative and floral extracts (Fig. 1A, B, C and D). Differences observed were of two general types--1) a few of the flavonoid component spots present in the hybrid patterns were found in only one of the two parental patterns; and 2) some flavonoid component spots were unique to the hybrid and therefore missing from both parental patterns. These observed differences in flavonoid composition among the three clones might lend insight into the inheritance and expression of the genes controlling their synthesis in Zygocactus. The absence of a compound from one parent but present in the hybrid could mean that a dominant allele(s) for the flavonoid existed in the parent which had the demonstrated compound. The flavonoid represented by vegetative spot 25 and floral spot 11 therefore were most likely inherited from female parent 1178 (Tables 1 and 2). At least two possible explanations could account for the appearance of hybrid compounds, i.e., those compounds found in the hybrid but missing in both parental types. The hybrid could have inherited as a result of the recombinational process one recessive allele for the particular trait, i.e., flavonoid, from each of the parental typess which would result in the hybrid becoming homozygous recessive for that gene pair and having a flavonoid not present in either parent. Secondly, the hybrid might inherit from each heterozygous parent an allele from a regulatory locus that is not expressed, i.e., recessive in each parent. The inheritance of these two normally recessive alleles by the hybrid might result in derepression of the expression of another gene pair controlling the synthesis of the new hybrid flavonoid. The dominant regulatory allele also present in both heterozygous parentals would have caused a repression of the synthesis of the hybrid flavonoid in the parentals.

The results obtained with paper chromatographic analysis indicated that most of the flavonoids (aglycones or the aglycone part of the flavonoid glycosides) produced by Zygocactus were flavonols (Tables 6 and 7). Several also appear to be dihydro flavonols and isoflavones. These results are consistent with previous studies on Cactaceae. For example, flavonols were discovered to be the dominant class of flavonoids in Opuntia (Miller and Bohn, 1982) and Echinocereus (Breckenridge and Miller, 1982).

The results reported in this study of Zygocactus are consistent with previous reports which indicated that inheritance of flavonoids is normally additive although occasionally either some parental constituents were missing or some additional hybrid compounds are present (Harbourne, 1975). All flavonoids present in either both or one of the parents were found in the hybrid consistent with the additive nature of flavonoid inheritance--i.e., no parental constituents were missing in the hybrid. A few flavonoids, however, were present in the hybrid only (vegetative spots 11 and 14; floral spots 9 and 10). The presence of these new compounds, as previously stated, could be due to a breakdown in the hybrid of the regulatory mechanisms responsible for the repression of certain flavonoid genes in the parents (Harborne, 1971).

Comparisons of chromatographic profiles indicated that most of the flavonoids present in Zygocactus were glycosides because the composite patterns of hydrolyzed extracts were significantly different from those obtained from unhydrolyzed extracts. The few flavonoid spot components which remained unaltered in color and R_f value after hydrolysis and chromatographic analysis were presumably aglycones. Acid hydrolysis of the extracts also revealed that the differences in flavonoid composition among the three cultivars were not due to different sugar glycosides attached to the same aglycone which would have resulted in the same R_f value for hydrolyzed flavonoid. Instead it appears that each cultivar possesses flavonoid glycosides with different aglycones because chromatographic analysis of the acid hydrolyzed extracts produced a significantly new pattern.

A reduction in the number of flavonoid spot components after acid hydrolysis of the vegetative material (Fig. 1A and C) was observed. Several different glycosidic sugar attachments to the same aglycone provides a possible explanation for this situation. Hydrolysis of the glycosides would result in the release of only one aglycone which would appear as a single spot in chromatographic analysis.

Floral chromatographic patterns of acid hydrolyzed extracts however, had additional flavonoid spot components (Fig. 1B and D) which might be explained as follows: several different unhydrolyzed flavonoid glycosides with similar R_f values and colors; i.e. appearing as single spots in chromatographic analysis would, upon hydrolysis yield several different aglycones each having different R_f values and color appearance when chromatographed.

Determinations of the flavonoid class from the wave length maximum of Band I and Band II were consistent with the preliminary assignment of those spots to a flavonoid class based upon their color appearance and R_f value. Floral spot numbered 4 was almost identical to the known flavonoid rutin (Fig. 4 and Table 13) in spectral profile as well as R_f values, sugar linkages, color reaction and the presence of a 5-OH group substitution. The identity of floral spot 4 as rutin requires confirmation by nuclear magnetic resonance or other techniques.

Although the individual flavonoids of Zygocactus were not identified in the study reported here, the patterns produced by two dimensional chromatographic analysis were sufficiently different to distinguish three closely related horticultural varieties. Such data can frequently be used directly in taxonomic studies without further chemical identification (Harborne, 1975). Our results with chemical analysis of parents and hybrids are similar to the chromatographic analyses of ferns by Smith and Levin (1963) which showed it was possible to identify hybrids derived from two and three parental species using spot pattern data and genome analysis. The clarity of their chromatographic data in elucidating the parental origin of the various natural hybrids has been quoted as one of the classical examples of additive inheritance of chemical characters in plants. Our results are consistent with and lend further support to that concept.

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